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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Kensil	Confirmation No.:	2171
Serial No.:	09/760,506	Art Unit:	1636
Filed:	January 12, 2001	Examiner:	Qian, Celine X.
For:	INNATE IMMUNITY-STIMULATING COMPOSITIONS OF CPG AND SAPONIN AND METHODS THEREOF	Attorney Docket No:	8449-153

DECLARATION OF DR. RAPHAEL CLYNES UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, RAPHAEL CLYNES, do declare and state that:

1. I am a citizen of the United States of America.

2. I presently hold the positions of Assistant Professor in the Department of Microbiology and Department of Medicine at Columbia University. Further positions I have held are set forth in my *curriculum vitae*, attached hereto as Exhibit 1.

3. I received the degree of Bachelor of Sciences in Biology from M.I.T. in 1983. I received the joint degree of Medical Doctor and Doctor of Philosophy from the State University of New York in 1990.

4. My education, technical experience and professional activities, honors and awards, and list of recent publications are set forth in my *curriculum vitae*, attached hereto as Exhibit 1. I have worked extensively in the areas of tumor immunity and autoimmunity.

5. I understand that the pending claims are directed to a method of treating cancer by administering a composition comprising a *Quillaja saponaria* saponin (or a chemically modified form thereof), where the composition does not contain a vaccine antigen. I have been informed and believe that claims of the above-identified patent application (the "'506 application") are subject to a rejection by the Examiner based at least in part upon the Examiner's contention that inhibition of tumor growth by administration of a *Quillaja saponaria* saponin other than in the vicinity of the tumor is not enabled.

6. The following experiments were carried out by me or under my supervision. These experiments demonstrated that administration of a substantially purified saponin, QS-21, in the absence of a vaccine antigen, can stimulate innate immunity so as to inhibit tumor growth distant from the site of administration of the saponin.

7. First, the antitumor effects of QS-21 were evaluated in a B16F10 melanoma model/lung metastases mouse model. Beginning on day -1, three C57BL/6 mice were injected subcutaneously with phosphate buffered saline ("PBS") or 10 μg QS-21 three times a week for 2 weeks. The mice were injected intravenously with 2×10^5 B16F10 cells on day 0. On day 14, after tumor cell injection, the mice were sacrificed. The lungs were removed and fixed in 10% buffered formalin.

8. Exhibit 2, photographs of the lungs, shows the result of the above described experiment. Visual inspection of the lungs revealed that administration of QS-21 resulted in fewer tumor nodules.

9. The B16F10 studies described in paragraphs 7-8 above demonstrated that administration of a substantially purified saponin, QS-21, in the absence of a vaccine antigen, can stimulate innate immunity so as to inhibit tumor growth distant from the site of administration of the saponin.

10. In view of the foregoing, I conclude, and others skilled in the art would also conclude, that QS-21, in the absence of a vaccine antigen, can inhibit cancer, when administered other than in the vicinity of the tumor.

11. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 7/12/05

RAPHAEL CLYNES

Attachments:

Exhibit 1: *Curriculum Vitae* of Raphael Clynes

Exhibit 2: Photographs of lungs removed from mice injected with B16F10 cells

RAPHAEL A. CLYNES, M.D., PH.D.

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Tel:(212) 305-5289 FAX:(212) 305-1392, rc645@columbia.edu
dob: 3/5/61 New York, NY

Assistant Professor, Department of Medicine and Microbiology, Columbia Univ.
Attending Physician, Division of Oncology

Positions

Assistant Professor, Attending Physician 9/99-
Co-Director, Immunology Program, Columbia University Irving Cancer Center
Department of Medicine and Microbiology
Columbia University

Clinical Instructor, 1998-9
Clinical Immunology Service
Memorial Sloan-Kettering Cancer Center, NY, NY

Research Associate/Associate Physician 1996-
Laboratory of Jeffrey V Ravetch, Rockefeller University, NY, NY

Education

Fellowship in Hematology/Oncology, 1992-8
Memorial Sloan-Kettering Cancer Center, NY, NY

Internship and Residency in Internal Medicine 1990-2
Barnes Hospital, St Louis, MO

MD 1990
State University of New York at Stony Brook

PhD in Biochemistry 1990
Laboratory of Kenneth B Marcu, SUNY at Stony Brook

BS in Biology 1983
Massachusetts Institute of Technology

NIH Funding

NIH/NCI R01 CA94037-01 (12/01-12/05)
"FcR Enhancement of Antigen Presentation; Implications for Vaccine Development"

DOD/Breast Cancer Idea Award BC981021 (1999-2002)
"Cytotoxic Mechanisms of Antitumor Antibodies"

NIH/NIDDK Clinical Investigator Award K08 DK02468 (1996-2001)
"Fc Receptors in Autoimmunity and Glomerulonephritis"

NIH/NIAMS Small Grant Program R03 AR 45764-01 (1999-2001)
"Identification of Fc Receptor Effector Cells in Autoimmunity"

NIH/NHLBI: Program Project P01AI50514-01 Project leader (project 3) (11/01-11/05)
"Fc Receptors in Asthma Pathogenesis".
P01 " Asthma and Allergic Diseases Research Center"

Other external peer-reviewed funding:
Empire Clinical Research Investigator Program 2005
National Juvenile Diabetes Research Foundation (Program Project, Project 1) 2004
Speaker Award of the New York Academy of Sciences 2001
Kimmel Scholar Cancer Award 2001
Cancer Research Institute 2000
Lupus Clinical Scholar/Arthritis Investigator Award 1999

Internal peer-reviewed funding
NIH/CFAR Pilot Award 2004
NIDDK/DERC Pilot Award 2004
Avon Cancer Center Award 2000

Awards
New York State Regents Scholar of Medicine 1983
Alpha Omega Alpha 1989
American Cancer Society Clinical Oncology Fellow 1992
MSKCC Clinical Scholar 1993-6
Lupus Clinical Scholar/Arthritis Investigator Award 1999
Gail Williams Biomedical Scholar Award 2000
Cancer Research Institute Investigator Award 2000
Charles Carrington Prize in Molecular Mechanisms of Disease 2000
Speaker Award of the New York Academy of Sciences 2001
Kimmel Scholar Cancer Award 2001
The Harold and Golden Lamport Award for Excellence in Basic Science Research 2004
Empire Clinical Research Investigator Program 2005-2007

Medical Certifications/Licensures
Board Certified Diplomate in Internal Medicine 1994
Board Certified Diplomate in Medical Oncology 1995
Board Certified Diplomate in Hematology 1997

Other Professional Activities

Study Sections:
Department of Defense Breast Cancer, Immunological Sciences Study Section 2003
Arthritis Foundation Review Committee, Clinical Immunology Study Section 1999-2002
Austrian Science Fund, Ad hoc Reviewer 2003
NIH/IMM-A Study Section Member 2004

Journal Review:
Associate Editor: Journal of Clinical Investigation 2003
Ad hoc Reviewer: Blood, Cancer Research, Science, Nature, Nature Immunology,

Journal of Biological Chemistry, Vaccine, Journal of Clinical Oncology, Journal of Clinical Investigation, International Immunology

Teaching:
1999- Attending Rounds, Division of Oncology, Columbia-Presbyterian Hospital
1999- Lecturer, Medical School Immunology Lecturer 1999- , Lecturer,
2001- Graduate School Immunology, Columbia University, College of Physicians and Surgeons, Lecturer
2001- Medical Oncology Fellowship Lecture Series, Lecturer Immuntherapeutics in Cancer
2005 Invited Lecturer, Antibodies and Tumor Immunity, Principles of Immunotherapeutics, Alan Houghton, Course Director, MSKCC/Cornell University
Thesis Committees: Rachel Liberatore, Bao Vong, Alex Banks, Miriam Baumgarten, Ken Flanagan, David Sayah
Qualifying Examination Committee Microbiology 2000, 2005
Qualifying Examination: Miriam Baumgarten, Martha Neugue
Organizer Immunology Program Visiting Immunology Lecture Series 2002-

Invited Speaker
Guest Lectures
1999 Albert Einstein, Boston University, NYU, UC Irvine, Mt. Sinai, University of Minnesota, University of Utah
2000 Mederex Inc., Washington University
2001 Chiron Corporation
2001 University of Florida, Johns Hopkins University
2002 Albert Einstein, Stanford University
2003 University of Miami
2004 Johns Hopkins University

Scientific Meetings
2000 Keystone Symposia on Tumor Immunology
2002 1st Annual Symposium on Anti-Receptor Signaling Human Neoplasia
2002 International Congress on Antitumor Antibodies
2003 Keystone Symposia: Antibody-Based Therapeutics for Cancer
2003 Keystone Symposia: Immunobiology and Cell Biology of Dendritic Cells
2003 International Congress on Antitumor Antibodies
2003 2nd International Workshop on Non-Hodgkin's Lymphoma
2003 American Society of Hematology (ASH) Making the Connection Between Basic Science and Optomized Clinical Utility
2004 International Congress on Antitumor Antibodies
2005 Keystone Symposia: Antibody-Based Therapeutics for Cancer
2005 Inaugural Joint American-Israeli Conference on Cancer

Advisory Board Meetings
2002 New Advances in Biological Therapy: Applications in Oncology and Immunology (Genentech).
2002 Role of Interleukin-2 in Enhancing Therapeutic Efficacy of Monoclonal Antibodies (Chiron).
2003 Chiron Biopharmaceutical Advisory Board
2003 and 2004 Update on Erbitux (Imclone)
2004 Xencor Clinical Advisory Board

Lymphocyte Transformation

1) **Clynes, R.**, Wax, J., M. Potter and K.B. Marcu. Experimental models of transformation and progression. Mechanisms of B cell Neoplasia 1987, Basel Institute of Immunology and Hoffman-LaRoche Co., 323-344. (1987)
2) Primi, D., **Clynes, R.**, Jouvin-Marche, E., Marolleau, J.P., Barbier, E., P.A. Cazenave and K.B. Marcu. Rearrangement and expression of T cell receptor and immunoglobulin loci in immortalized CD4-CD8- T cell lines. Eur. J. of Immunol. 18:1101-1109 (1988)
3) **Clynes, R.**, Wax, J., Stanton, L.W., Smith-Gill, S., M. Potter and K.B. Marcu. Rapid induction of IgM-secreting plasmacytomas by pristane and an immunoglobulin heavy-chain promoter and enhancer-driven c-myc/v-Ha-ras retrovirus. Proc. Natl. Acad. of Sci. USA 85:6067-71. (1988)
4) **Clynes, R.**, Stanton, L. Wax, J., Smith-Gill, S., M. Potter and K.B. Marcu. Synergy of an IgH promoter-enhancer driven C-myc/v-Ha-ras retrovirus and pristane in the induction of murine plasmacytomas. Mechanisms of B Cell Neoplasia 1988. Curr. Top. in Microbiol. and Immunol. 141:115-125. (1988)
5) Mock, B., Wax, J., **Clynes, R.**, K.B. Marcu and M. Potter. The genetics of RIM-induced plasmacytomagenesis. Mechanisms of B-cell Neoplasia 1988. Curr. Top. in Micriobiol. and Immunol. 125-128. (1988)
6) Primi, D., Jouvin-Marche, E., **Clynes, R.**, Marolleau, J.P., Gris, C., K.B. Marcu and P.A. Cazenave. Dynamics of immunoglobulin and T-cell receptor genes recombinations during lymphocyte development. in The Immunology of the Fetus. CRC Press, Inc.,(1989)

Fc Receptors

7) Takai, T., Li, M., Sylvestre, D., **Clynes, R.** and J. Ravetch. FcR γ chain deletion results in pleiotrophic effector cell defects. Cell 76:1-10 (1994)
8) **Clynes, R.** and J.V. Ravetch, Cytotoxic antibodies trigger inflammation through Fc receptors. Immunity, 3:21-26 (1995)
9) **Clynes, R.**, Sylvestre, D., Ma, M., Carroll, M. and J. V. Ravetch, IgG mediated inflammatory responses develop normally in complement deficient mice. JEM 184; 2385-2392. (1996)
10) Vasovic, L., Dyall, R., **Clynes, R.**, Ravetch, J.V. and J. Nikolic-Zugic, Synergy between an antibody and CD8+ cells in eliminating an established tumor. Eur. J. of Immunol. 27;368-3. (1997)
11) Sutterwala, F., Noel, G., **Clynes, R.** and D.M. Mosser, Selective suppression of Interleukin-12 induction following macrophage FcγR receptor ligation. JEM, 185; 1-9. (1997)
12) **Clynes, R.**, Dumitru, C. and J.V. Ravetch, Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. Science, 279, 1052-4 (1998)
13) **Clynes, R.**, Yoshizumi, T., Moroi, Y., Houghton, A.N., and J.V. Ravetch, Fc Receptors are required for passive and active immunity to melanoma. PNAS, 95 (2) 652-656. (1998)
14) Rotman HL, Daly TM, **Clynes R**, Long CA, Fc receptors are not required for antibody-mediated protection against lethal malaria challenge in a mouse model J Immunol 161:1908-12 (1998).
15) Weber LW, Bowne WB, Wolchok JD, Srinivasan R, Qin J, Moroi Y, **Clynes R**, Song P, Lewis JJ, Houghton AN Tumor immunity and autoimmunity induced by immunization with homologous DNA.J Clin Invest 102:1258-1264 (1998).

16) Yuan, R., **Clynes, R.**, Oh, J., Ravetch, J.V., and M.D. Scharff, Antibody-mediated protection to *C. neoformans* is dependent on distinct Fc receptors and IgG subclass. JEM, 187, 641-648 (1998)
17) Jankovic, D., Cheever, A., Kullberg, M., Wynn, T., Yap, G., Caspar, P., Lewis, F., **Clynes, R.**, Ravetch, J.V. and A. Sher, CD4+ T cell mediated granulomatous pathology is down-regulated by a B lymphocyte-dependent mechanism requiring Fc receptor signaling. JEM, 187, 619-629 (1998)
18) **Clynes R,** Maizes JS, Guinamard R, Ono M, Takai T, Ravetch JV Modulation of Immune Complex-induced Inflammation In Vivo by the Coordinate Expression of Activation and Inhibitory Fc Receptors J Exp Med 189:179-186. (1999)
19) Dyall, R., Vasovic, L., **Clynes, R.** and J. Nikolic-Zugic, Cellular Requirements for the monoclonal antibody-rejection of established tumors Eur J Immunol, 29:30-7 (1999)
20) Liliane Fossati-Jimack, Luc Reininger, Yves Chicheportiche, **Raphael Clynes**, Jeffrey V. Ravetch, Tasuku Honjo, and Shozo Izui, High Pathogenic Potential of Low-Affinity Autoantibodies in Experimental Autoimmune Hemolytic Anemia, J. Exp. Med. 190: 1689-1696 (1999).
21) **Clynes RA,** Towers TL, Presta LG, Ravetch JV, Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med, 6:443-446 (2000).
22) Hatzivassiliou, G, Miller, I, Takizawa, J, Pulivarthi, R, Palanisamy, N, Iida, S, Tagawa, S, Russo, J, Neri, A, Cattoretti, G, **Clynes, R** , Mendelsohn, C, Chaganti, RSK, and R Dalla-Favera, IRTA-1 and IRTA-2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy, Immunity 4:277-89 (2001).
23) Rafiq, K., Bergtold, A., and **R. Clynes,** Immune complex-mediated antigen presentation induces tumor immunity, JCI 110:1 (2002)
24) J. Trcka, Y. Moroi, **R. Clynes**, S. Goldberg, A. Bergtold, M-A Perales, M. Ma, C. Ferrone, M. C. Carroll, J. V. Ravetch and A. N. Houghton, Redundant and Alternative Roles for Activating Fc Receptors and Complement in an Antibody-Dependent Model of Autoimmune Vitiligo Immunity 16 861-868 (2002).
25) Curcio C, Di Carlo E, **Clynes R,** Smyth MJ, Boggio K, Quaglino E, Spadaro M, Colombo MP, Amici A, Lollini PL, Musiani P, Forni G, Nonredundant roles of antibody, cytokines, and perforin in the eradication of established Her-2/neu carcinomas, JCI, 111: 1161-1170 (2003).
26) **Clynes, R.,** Calvani, N., Croker, B.and H. Richards, Modulation of the immune response in pristine-induced lupus by activating and inhibitory Fc receptors, (Clinical and Experimental Immunology, in press)
27) Bergtold, A, and **R. Clynes,** Cell Surface Recycling of Internalized Antigen Permits B Cell Priming by Dendritic Cells (Immunity, conditionally accepted pending revisions).
28) Desai, D., Bergtold, A., and **R. Clynes**, FcRII Functionally Inhibits Immune complex-Mediated Antigen Presentation, (Manuscript in Preparation).
29) Bergtold, A., Gavhane, A. and **R. Clynes**, Modulation of Lupus Nephritis by FcR Expression on Hematopoietic Cells, (manuscript in preparation).
30) Ogryzlo-Harbers, S., Desai, D., Gavhane, A., and **R. Clynes,** Antibody-mediated cross-presentation of self antigen induces loss of tolerance and autoimmunity, (manuscript in preparation).
31) Liang, B., Vignali, D. and **R. Clynes,** LAG-3 Inhibits Dendritic Cell Function through MHC II-mediated recruitment of FcRgamma. (manuscript in preparation).
32) Taylor, C., Hershman, D. and **R. Clynes,** Induction of Humoral anti-HER2 responses during trazustumab therapy (manuscript in preparation).

33) Langer, B., Desai D., Schmidt, A-M., and **R. Clynes,** RAGE Provides Co-stimulatory Activity in T cell Priming and Transplant Rejection (manuscript in preparation).
34) Constantinescu, A., Catalano, G. and **R.Clynes,** Fc receptor-mediated dendritic cell activation induces bystander cell activation through membrane-bound TNF (manuscript in preparation).

Invited Reviews

1) **Clynes, R.** and J.V. Ravetch, Divergent roles of Fc receptors and complement in vivo. Annual Review of Immunology, 16:421-432, (1998)
2) **Clynes R.**, Fc Receptors and Antibody-Mediated Immunotherapy of Lymphoma, Biological Therapy of Lymphoma (2002).
3) **Clynes R.**, Overview of the Fc receptors: their function in ADCC, Biological Therapy of Lymphoma (2002).
4) **Clynes, R.** and Dalla Favera, R., Human Fc Receptor Homologues; Novel B Cell Restricted Inhibitory Immunoreceptors, Trends in Immunology, (in preparation)
5) **Clynes, R., Mechanisms and clinical activity of antitumor antibodies,** ***General Principles of Tumor Immunotherapy: Basic and Clinical Applications of Tumor Immunology***" Kluwer Academic Publishers – Springer, (in preparation)
6) **Clynes, R.** Immune complexes as therapy for autoimmunity, J Clin Invest. 2005 Jan;115(1):25-7.

Existing Support: CLYNES, RAPHAEL A. (principle investigator)

ACTIVE
P01AI50514-01 9/01-9/05
Project leader (project 3) $180,000/yr (Indirect $114,300)
"Fc Receptors in Asthma Pathogenesis".
P01 " Asthma and Allergic Diseases Research Center" Director Chris Schindler M.D.. Ph.D.
The major goals of this project are determine the contributions of Fc receptors to airway inflammatory responses in both priming and effector phases.

ACTIVE
R01 NCI CA94037-01 8/02-8/06
FcR Enhancement of Antigen Presentation; $200,250/yr direct ($127,159indirect)
Implications for Vaccine Development

ACTIVE
Juvenile Diabetes Research Foundation 7/04-7/06
RAGE Blockade and Diabetes $100,00/yr

ACTIVE
Center for AIDS Research Developmental Awards 7/04-7/05
Developmental Award 2003: Fc receptors and HIV Immunopathogenesis
$50,000/yr

ACTIVE
Diabetes Endocrinology Research Center Pilot Award 9/04-9/06
Pathogenic Role of Islet Cell Autoantibodies in Diabetes
$50,000/yr

ACTIVE

Empire Clinical Research Investigator Program 7/01/05-7/01/07
FcR-mediated Immunotherapeutic Mechanisms $60,000/yr

PENDING AWARDS

Imclone Systems
Identification of an ADCC Signature in vivo
(submitted with Charles Powell)
$100,000/yr

Merck Company
Identification of an ADCC Signature in vivo
(submitted with Charles Powell)
$100,000/yr

RECENTLY REVIEWED

R01 DK070999 Reviewed December 2004
Pathogenic role of islet cell Abs in autoimmune diabetes
Priority Score: 213 $250,000/yr
Planned Rubmission Date 11/05

Completed Projects

EXPIRED
Lupus Scholar Award/ 1/00-1/06
Arthritis Investigator Award
National Arthritis Foundation $60,000/yr direct costs
Identification of FcR Effector Cells in Autoimmunity
The major goals of this project are to determine the contributions of specific FcR positive lineage cells to immune complex inflammatory responses.

EXPIRED
Investigator Award 7/00-7/04
Cancer Research Institute $50,000/yr direct costs
Cytotoxic Mechanisms of Tumor Specific Antibodies
Determination of the role of fas in ADCC in vivo.

EXPIRED
Speaker Fund for Biomedical Research 7/01/-7/04
FcR Enhancement of Antigen Presentation; $100,000/yr
Implications for Vaccine Development

EXPIRED
Kimmel Cancer Research Award 7/01-7/03
FcR Enhancement of Antigen Presentation; $100,000/yr
Implications for Vaccine Development

EXPIRED

K08 DK02468 12/1/96-11/30/01

NIH/NIDDK $90,000/yr direct cost

FcγR in Autoimmune Glomerulonephritis and Autoimmunity

The major goals of this project are to study the role of FcγR in clearance of particulate and soluble immune complexes and to characterize disease development in Fc□R -/- MRL and NZB/NZW mice.

EXPIRED

1RO3AR45764-01 11/98-11/01

NIH/NIAMS $50,000/yr. direct cost

Identification of FcR Effector Cells in Autoimmunity

The major goals of this project are to determine the contributions of specific FcR positive lineage cells to immune complex inflammatory responses

EXPIRED

BC981021 10/99-9/02

DOD/Army Idea Award $70,000/yr direct cost

Cytotoxic Mechanisms of Tumor Specific Antibodies

The major goals of this project are to determine the molecular pathways mediating ADCC in vivo.

Training Record

		Period	Degree	School	Project
Former Postdocs	Rafiq, K.	00-01	PhD, 00	Catholic U., Belgium	IC-Mediated Tumor Immun
Current Postdocs	Desai, D.	02-	PhD, 98	U. of Tennessee	FcRII-Mediated Ag Presenta
	Liang, B.	03-	PhD, 00	NY Med Coll.	HER-2 Targeted Ab-Therap
Current Predocs	Ogryzlo-Habers, S	03-	BS, 00	U of Toronto	Islet cell Abs and Diabetes
	Bergtold, A.	01-	BS, 97	Mt Holyoke	DC activation of B cells
	Catalano, G.	03-	BS, 01	SUNY Buffalo	FcRII and Tolerance

Current Staff

	Position	Funding Mechanism
Raphael Clynes	PI	R01/P01/JDRF
Dharmesh Desai	Research Scientist	RAGE/JDRF + unfunded
Bitao Liang	Post-doctoral Fellow	Pulmonary Training Grant
Nina Shah	Clinical Fellow	Empire Clinical Award
Clare Taylor	Staff Associate	P01
Geoffrey Catalano	Grad Res Asst	Immunology Training Grant
Stephanie Ogryzlo-Harbers	Grad Res Asst	DERC/Diabetes Award
Amy Bergtold	Grad Res Asst	CFAR/R01
Matthew Downey	Res Technician	unfunded
Judy Vasquez	Animal Tech (part-time)	R01